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**INTRODUCTION**

Finding resources is an essential task for the growth, reproduction and development of every organism. Therefore, the efficiency of an animal’s searching behavior is fundamental to its survival and fitness. There are multiple morphological, physiological and behavioral adaptations that allow individuals to efficiently locate resources like food, mates, or refugia, among others. But first and foremost, animals must be able to detect cues that will allow it to pinpoint the location of potential resources through various sensory modalities (Bell, 2012). Vision, for example, is used by female zebra fishes *Danio rerio* to recognize their conspecifics, with whom they tend to aggregate and form shoals to increase the chances of mating, increase protection from predators and to locate food (Pitcher, 1986; Engeszer *et al.*, 2007; Gerlai, 2014; Dreosti *et al.*, 2015; Nunes *et al.*, 2020). Crayfish, on the other hand, relies basically on chemical signals for mating and locating shelter (Moore, 2019). In most cases, however, animals probably use cues from various sensory modalities to search and locate resources. For example, octopuses can integrate information from both chemical and visual cues during food search and selection, e.g., while chasing prey (Maselli *et al.*, 2020).

In bats, finding resources is often linked with the use of sound in the form of echoes from self-produced vocalizations (i.e. echolocation), which is often considered as their most important sensory modality (Griffin, 1958; Dechmann and Safi, 2005; Gillam and Fenton, 2016). Echolocation certainly plays a role in searching for food, especially in insect-hunting bats (Thies *et al.*, 1998; Schnitzler and Kalko, 2001), but is also very relevant for spatial navigation and orientation (Griffin, 1958; Schnitzler and Kalko, 2001; Schnitzler *et al.*, 2003; Nelson & MacIver, 2006; Neuweiler, 2000; Thomas, Moss, & Vater, 2004). Sound is also known to greatly aid during the location of roost-sites, but primarily in the form of acoustic cues or signals emitted by conspecifics which have already located a suitable roost site (Vaughan and O’Shea, 1976; Ruczynski *et al.*, 2007, 2009; Chaverri and Gillam, 2010). So far it is not known whether echolocation is also used for location of roosts, but given its role during small-scale orientation (e.g. Schnitzler *et al.*, 2003), it is likely to play a large role during this process. Yet, bats’ detection range based on echolocation can be limited given the high attenuation rates of the high-frequency calls typically used by bats (Bradbury and Vehrencamp, 2011; Eklöf, 2003). Therefore, other senses or sensory cues may complement echolocation in the detection of suitable resources, like vision (Eklöf, 2003; Ruczynski *et al.*, 2007; Ruczynski *et al.*, 2011; McGowan and Kloepper, 2020) or smell (Hessel and Schmidt,1994; Kalko *et al.*, 1996; Von Helversen *et al.*, 2000), primarily given the relatively large areas over which bats must locate roosts. The combination of cues from different sensory modalities may increase the bats’ success in finding roosts.

A bat species that is required to constantly locate new roosts sites is Spix’s disc-winged bat *Thyroptera tricolor*, an insectivorous bat occurring in lowland Neotropical forests from southern Mexico to southeastern Brazil (Wilson and Findley, 1977) that forms very stable social groups of 2 to 12 individuals (Vonhof and Fenton, 2004). This species roosts in the unfurled leaves of plants in the order Zingiberales, that typically grow in secondary forests and clearings; this implies that there is large spatial variability of this resource (Levey, 1988). The leaves remain in their ideal tubular shape for bat roosting for very short periods of time (approximately 1 day), which means that *T. tricolor* probably also needs to change roosts during the day (Findley and Wilson, 1974; Vonhof and Fenton, 2004). Therefore, individuals must constantly locate new roosts sites in areas that span approximately 0.19 ha (Vonhof *et al.*, 2004). Several studies show that an exchange of social calls is critical for rapid location of new roost-sites when a group member has already found and occupied a suitable tubular leaf (then several citations should go here); however, how individuals locate new roosts without the aid of conspecifics is unknown. *T. tricolor* emits low-intensity echolocation calls of dominant frequencies ranging from 45-47 kHz, which are barely detected with a microphone at a distance of 1 m from the bat (Fenton *et al.*, 2000). Thus, considering the extremely short range over which echolocation calls could aid in roost location, coupled with the fact that the background clutter from other plants and leaves all around the roost makes it even harder to find (Eklöf, 2003), it is possible that visual cues may be important for disc-winged bats when searching for roosts.

In this study we aim to contribute to a more comprehensive understanding of *T. tricolor*’s sensory world during the process of roost finding. Roosts provide critically important resources for bats, including protection from predators, and *T. tricolor* faces an important constant challenge that the tubular leaves they use for roosting may fully open during the daytime, rendering group members vulnerable to predation. Finding a new roost thus becomes extremely urgent, particularly during the daytime when bats are approximately 10 times more likely to be hunted by diurnal predatory birds (Speakman *et al.*, 1994). In this situation, echolocation calls might not provide enough information over longer distances and in a timely manner; thus, we predict that bats will primarily rely on vision for this task. Our results represent a significant contribution to our understanding of the sensory ecology of bats, more specifically in a task that is vital for their survival, i.e. the location of suitable roost-sites, that nonetheless remains a very poorly studied topic to date.

**METHODS**

The use of vision in *T. tricolor* for roost finding was investigated based on a set of experiments performed in both daylight and darkness. The daylight experiments took place in a 5 weeks period, from November 6 through December 7 2019, in a farm (8°38′N, 83°05′W; 93.6 ha) on road 14 Golfito-Rio Claro, in southwestern Costa Rica . This site consists of a field station surrounded by a matrix of primary and secondary wet tropical forest and agricultural lands. *Heliconia imbricata* and *Calathea* *lutea* are abundant in the understory, being the main roosting resource for *T. tricolor* in the site (Buchalski *et al.*, 2014). The night experiments took place between the 24th and the 29th of January 2020 in Barú Biological Station, Puntarenas Province, a private 320 ha coastal lowland of the Pacific slope in Costa Rica (9°81’ N, 84°81’ W) (Johnson, 2005). We searched for groups of *T. tricolor* in areas with high abundance of plants used as roosts.In order to capture a group of bats, all the potential leaves that were in the furled stage were examined with a telescopic mirror. If a group of bats was detected, the top of the leaf was pinched and closed, so that the animals could not fly away. Then the leaf was inserted in a plastic bag (20cm x 1m) and the bats were gently directed out by closing gradually the leaf behind them. All bats inside the leaf were promptly transferred from the plastic bag to the cloth bag and taken back to the field station. All the individuals found in the same leaf were considered to be part of the same group (Vonhof et al. 2004; Chaverri 2010). Once at the field station, bats were identified with an HPR Tag Reader (Biomark, Boise, Idaho), sexed, aged and their forearm length measured.

In order to test the relative importance of two sensory modalities for roost finding, sound and vision, we conducted experiments within a flight cage (2,5 x 3,5 x 5,5 m), one made of saran shade cloth, which was used during the daytime, and another made of double-walled cloth to reduce the amount of artificial light for our nocturnal experiments. Two Ultrasonic Omnidirectional Dynamic Speakers (Vifa, Avisoft Bioacoustics, Glienike/Nordbahn, Germany) were positioned inside the flight cage on tripods at two meters distance one from each other. A furled leaf of *Heliconia* sp*.* or *Calathea lutea*, taped on a tripod, was then placed between the two speakers. The leaf was replaced every day with a new one. An UltraSoundGate Player 216H (Avisoft Bioacoustics), controlled by Avisoft Recorder software (Avisoft Bioacoustics), was used to broadcast playback sounds that would allow us to mask calls from bats. In order to reach maximum sound levels, the latter was attached to an external power supply, consisting of a set of three batteries of 12V each connected in series (36V total).

For each bat, a total of 5 trials were performed during the daylight experiments, with the maximum duration of 5 minutes per trial. However, the first, explorative trial could last 1-2 minutes longer. The trial began when the bat started to fly and ended when the bat entered the leaf or when the maximum of five minutes was reached. After every experiment, the time (in seconds) required for the bat to enter the leaf was registered; if it did not enter, we registered a time of 600 s. The first trial, exploration (EXPL), always consisted of the bat flying without broadcasting any sound; its purpose was for the bat to explore and become familiar with the flight cage, in addition to identifying the presence of a suitable roost-site. Only bats who entered the leaf during the first exploration trial were used on subsequent experiments. When flights exceeded the five-minute limit, the bat was captured with a hand net. The following trials were randomized in order to minimize the effect of trial order in the bat’s behavior. In three of those trials (inquiry masking (IM), echolocation masking (EM) and broadband white noise (BBN)), the flights were conducted while broadcasting a playback, whereas the trial without masking (WM) was silent and used as a control. Playbacks started a few seconds before the bat was released and lasted the entire experiment. Moreover, since each bat performed the trials in sequence, the roost was moved about 1 m after each trial, in order to prevent the bat from finding the roost using spatial memory.

The masking sounds were created with the Seewave package in R (version 2.1.6; Sueur *et al.*, 2008), and they all have a duration of 30 seconds, sampling rate of 1 million hz and depth of 16 bits. When played, each sound was put in a loop mode so that the masking could last for the duration of the test. Furthermore, sound intensity was set to approximately 80 dB, measured at 1-meter distance from each speaker using (i.e. the distance at which the leaf was positioned) a sound level meter (Extech Instruments, New Hampshire, U.S.A.). Three types of playbacks were used in our experiments, inquiry masking white noise (IM), echolocation masking white noise (EM), and broadband white noise (BBN). IMisa sound intended to mask the frequencies of social calls that *T. tricolor* emits to inquire conspecifics about a roost’s location (inquiry calls). The frequencies of this type of signal range between 15 and 40 kHz, being at about 25.84 kHz the frequency with most energy (Chaverri *et al.*, 2010). The masking sound generated ranged from 0 to 45 kHz.EM is a sound in the frequencies of *T. tricolor*’s echolocation calls, whose frequencies range between 45 and 100 kHz, with the energy peak at about 47 kHz (Fenton *et al.*, 2000). This masking sound ranges from 45 to 200 kHz, to cover also the possible echoes returning from the echolocation call’s harmonics. BBN is a sound with wider frequencies that masks both inquiry and echolocation calls. This masking ranges from 0 to 500 kHz.

The night experiments were conducted in order to determine if a bat’s roost location abilities would suffer when no, or little, visual stimuli were available. These experiments consisted of only three trials: the first was exploration (EXPL), follows by BBN and WM, were randomized. We used an infrared camera (HD Pan/Tilt Wi-Fi Camera NC450) located within the flight cage to determine when the bat entered the roost.

After each experiment, we provided water and mealworms (*Tenebrio molitor*) to all individuals (Chaverri *et al.*, 2013). Also, during the night experiments, the bats were abundantly fed before they were tested in order to prevent them from searching for food instead of searching for a roost. After daylight experiments, the bats were gently released within the same leaf in which they were captured, or in a new one nearby. Bats from night experiments were released from the cloth bag near the capturing site.

All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling and care of mammals (Sikes et al., 2016) and the ASAB/ABS Guidelines for the use of animals in research. This study was conducted in accordance with the ethical standards for animal welfare of the Costa Rican Ministry of Environment and Energy, Sistema Nacional de Áreas de Conservación, permit no. SINAC-ACOPAC-RES-INV-008-2017 (Decree No. 32553-MINAE). Protocols were also approved by the University of Costa Rica's Institutional Animal Care and Use Committee (CICUA-42-2018).

Statistical analysis. We used Bayesian generalized linear mixed models in the R package MCMCglmm (Hadfield 2010) to evalute the effect of the different sensory inputs (predictor) in the time required for the bat to enter the leaf (response), including individual as random effect (random intercept). First, we ran a model with the response as a categorical variable to evaluate if there are detectable differences among sensory input categories. We then compared two models related to the number of sensory inputs available (as an ordinal variable). The first model represents a scenario in which the time to enter the roost decreases as a function of the addition of sensory inputs (ordinal categorical variable, 1st category = no inputs, 2nd = only visual or only vocal, 3rd = visual and vocal). The second model represents an alternative scenario in which any sensory input has a similar effect on roost entering time (two level categorical variable: 0 = lessen input, 1 = any input). We applied a model selection procedure based on the deviance information criterion (DIC) to determine the relative fit of competing models, including a null (intercept-only) model. All Bayesian mixed models were replicated three times using identical parameters, but randomly sampling the starting values from a Z distribution. We retained 9700 posterior samples for each model (chain length = 100 000, burn-in = 3000, thinning interval = 10). The Gelman-Rubin diagnostic (Gelma & Rubin 1992) was used to check for convergence between the three runs. We also inspected the trace and distribution of estimates between the three replicates and assessed the independence of successive sampled values (i.e. autocorrelation) of MCMC chains. We present effect sizes as mean posterior estimates as well as the highest posterior density (HPD) interval (e.g. 95% credible interval). Effect sizes in which credible intervals did not overlap with zero were considered to have an effect on the response variable.

**Results**

When evaluating the effect of treatment as a categorical variable only the lessen input treatment has a detectable effect. In this case it took longer for bats to enter the leaf compared to the control treatment (effect size = 427 s, 95% CI= -543.6- -279.1, Fig 1). This relationship was further explored by encoding treatment as a categorical variable representing two modes: the sum of sensory modalities or the presence/absence of sensory input. We found that a model representing sensory input as present (sound and or visual cues) or absent (lessen input, e.g. BBN at night) provides a better fit to the data. The model found that finding the roost tooks longer during the lessen input treatment compared to treatments with presence of any sensory input, regardless of the input modality (effect size = 512 s, 95% CI= -533.1- -269.8). We ran experiments on 33 individuals. However two individuals were excluded as only one treatment was properly assessed on them. For the remaining individuals, 10 were tested in two treatmens and 21 on three treatments.

mean_sd_and_violin_time_to_enter_by_treament

Fig. 1. Distribution (violin plots), mean and standard deviation (points and error bars) of time to entering roost by Spix’s disc-winged bats under five different treatments: control (IM), sound and vision (daylight without interference) sound (night), vision (daylight with broadband noise) and lessen input (night experiments with broadband noise).

**References**

Gelman, A., Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. Statistical Science, 7(4), 457–472.

Hadfield, J. D., & Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33(2), 1–22.